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Dynorphin-(1–13), an extraordinarily potent opioid peptide

(endorphin/[Leu]enkephalin/pituitary/neuropeptide/endogenous morphine-like peptide)

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ABSTRACT **We describe the opioid properties of a tridecapeptide, the sequence of which corresponds to the NH_2-terminal sequence of dynorphin, a novel porcine pituitary endorphin. It contains [Leu]enkephalin. In the guinea pig ileum longitudinal muscle preparation it is about 700 times more potent than [Leu]enkephalin. Its effects in this tissue are blocked completely by naloxone, but the apparent affinity of naloxone is $\frac{1}{13}$th that for blockade of [Leu]enkephalin or normorphine. In the mouse vas deferens, this peptide is 3 times more potent than [Leu]enkephalin. Well-washed rat brain membranes degrade the peptide rapidly, suggesting the presence of a membrane-bound degradative enzyme. The peptide displays considerable immunoreactivity in assays with antisera that have been used for the immunohistochemical localization of [Leu]-enkephalin. The remarkable enhancement of the potency of [Leu]enkephalin by the COOH-terminal extension -Arg-Arg-Ile-Arg-Pro-Lys-Leu-Lys-OH suggests new interpretations concerning the structure of opiate receptors and the function of the enkephalin pentapeptides.**

The first pituitary opioid peptide to be discovered (1, 2) had properties quite different from those of β-endorphin. It had a lower apparent molecular weight, was more basic, and had a more persistent effect in the guinea pig ileum bioassay. Also, its biologic activity (unlike that of β-endorphin) was resistant to destruction by cyanogen bromide. The final stages of purification presented special problems, which led to erroneous conclusions about composition and the presence of a blocked NH_2 terminus (3). Losses due to adsorption were particularly troublesome, and the material proved to be remarkably potent; consequently, the yield of purified product was much less than anticipated. However, by means of microsequencing technology (4, 5), we have now been able to determine the sequence of the first 13 residues.

To denote its extraordinary potency, the natural peptide has been named "dynorphin" (*dyn-* from Greek *dynamis* = *power*). Synthetic dynorphin-(1–13) proved to have approximately the same high potency as natural dynorphin. We describe here some properties of this synthetic tridecapeptide.

MATERIALS AND METHODS

Purification and Partial Sequence Determination of Porcine Dynorphin. Starting material was 100 g of melanotropin concentrate, the second oxycellulose adsorbate in commercial corticotropin production from porcine pituitary glands (6). The initial steps, with 25-g batches, have been described (2): extraction and back-extraction with butanol; separation from β-endorphin on Bio-Gel P-6; preparative reversed-phase high-performance liquid chromatography on C_{18} columns, first with a methanol gradient in trifluoroacetic acid and then with an acetonitrile gradient in Tris buffer at neutrality, followed by elution of the active material with trifluoroacetic acid. Every step was monitored by assay on the guinea pig ileum myenteric plexus longitudinal muscle preparation (7, 8).

Pooled material from four batches, obtained as above, was loaded on a Bio-Gel column (P-6/P-4, 4:1; 1.5 × 90 cm) in 0.1 M $(NH_4)_2CO_3$ at pH 8.7. Fractions (1.7 ml, 15 min) were tested for activity in the bioassay. The peak of slow-reversing activity (2) (fractions 65–75) was lyophilized and then further purified on CM-Sephadex (0.9 × 30 cm) equilibrated with 12.5 mM sodium borate at pH 11.0. The material was eluted with a 160-ml linear gradient from the starting buffer to 0.1 M phosphate buffer at pH 12.0. Fractions (2.0 ml; 15 min) were collected and assayed. Slow-reversing activity emerged between 94 and 106 ml. The two peak tubes, containing 50% of the activity, were pooled and desalted on Bio-Gel P-2 (1.5 × 90 cm) in *n*-butanol/acetic acid/water, 2:1:4 (vol/vol).

Finally, the desalted material was subjected to reverse-phase chromatography (high-performance liquid chromatography, C_{18} column) using a 10–50% acetonitrile gradient in 5 mM trifluoroacetic acid. The peak of biologic activity had no measurable absorbance at 280 nm. A rough estimate of quantity from absorbance at 225 and 215 nm indicated the presence of only a few micrograms of peptide, but the activity in the bioassay was equivalent to 334 nmol of normorphine (range, 189–525 in four tissue strips).

One-third of the material was dansylated (DNS), applied to a silica gel 60 H (Merck) thin-layer chromatography plate, and developed in methyl acetate/isopropanol/ammonia, 9:7:4 (vol/vol). Two fluorescent bands were observed with $R_F = 0$ (band 1) and $R_f = 0.20$ (band 2). Each band was extracted with methanol/pyridine/acetic acid, 1:1:1 (vol/vol), and demonstrated to be homogeneous by thin-layer chromatography in *n*-butanol/acetic acid/water, 4:1:5 (vol/vol) upper phase, with $R_F = 0.33$ (band 1) and $R_F = 0.51$ (band 2). After hydrolysis (6 M HCl, 16 hr, 105°C), material from each band was examined for dansylated NH_2-terminal residues by using reverse-phase high-performance liquid chromatography (C_{18} column; 45-min linear gradient; 5–50% acetonitrile in 10 mM Tris, pH 7.0; Varian fluorometric detector). Trace amounts of ε-DNS-Lys and di-DNS-Tyr were detected in the hydrolysate from band 1. The band 2 hydrolysate revealed only ε-DNS-Lys.

Opioid activity was destroyed by dansylation, so it was not possible to determine which peptide was the active principle by direct bioassay, and there was too little material to risk further loss in another purification step. Because it seemed likely that band 2 had a blocked NH_2 terminus, we decided to attempt microsequence determination on the remaining nondansylated material by the spinning cup procedure, as developed at California Institute of Technology (4, 5). In this method, the presence of NH_2-blocked peptide does not interfere in the sequence determination of a peptide with a free NH_2 terminus.

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Abbreviations: DNS, dansyl; IC_{50}, concentration for 50% inhibition.

With one-third of the total material, an unambiguous sequence was obtained for 13 residues (Fig. 1). The amount of peptide, estimated from the first few cycles, was about 400 pmol and, thus, the potency in the bioassay was approximately one-third of 334/0.400, or 267 times that of normorphine. Presence of the blocked contaminant was confirmed in the composition data (not shown), which gave distinctly noninteger ratios.

The tridecapeptide was synthesized for us by Peninsula Laboratories (San Carlos, CA) with a sequence corresponding to that of porcine dynorphin-(1–13): H-Tyr-Gly-Gly-Phe-Leu-Arg-Arg-Ile-Arg-Pro-Lys-Leu-Lys-OH. Purity was shown to be >98% in the following systems: (*a*) *n*-butanol/pyridine/acetic acid/water, 42:24:4:30 (vol/vol), cellulose plate, R_F = 0.54; (*b*) *n*-butanol/acetic acid/water, 4:1:5 (vol/vol) upper phase, silica gel plate, R_F = 0.05; (*c*) formic acid/acetic acid, pH 1.9, electrophoresis at 22 V/cm for 1 hr on Whatman 3MM, R_F = 1.40 with reference to picric acid. Composition was confirmed by amino acid analysis. We also confirmed the sequence of the synthetic tridecapeptide by analysis on the spinning-cup sequenator.

Two related peptides (referred to here as "register-shift peptides") were also synthesized by Peninsula Laboratories. These were the des-Arg^6 dodecapeptide from dynorphin-(1–13) and the Gly^6 tetradecapeptide derivative in which glycine is inserted between Leu^5 and Arg^6. Standards of purity were the same as noted above for the tridecapeptide.

Biologic Activity. Dilutions for all bioassays were made in methanol/0.1 M HCl, 1:1 (vol/vol); dilutions for radioimmunoassays were in 50 mM phosphate, pH 7.4/50 mM NaCl/0.1% bovine serum albumin/0.5% Triton X-100. Use of these solvents was dictated by our experience with losses to glass, plastic, and other surfaces during purification of the natural dynorphin and by experiments using ^{125}I-labeled dynorphin-(1–13).

The guinea pig ileum myenteric plexus longitudinal muscle preparation was used as described (7, 8). The concentration for 50% inhibition (IC_{50}) was determined by bracketing, with at least one concentration giving more than and one giving less than 50% inhibition of the electrically stimulated twitch, and interpolating on a logarithmic concentration/percentage inhibition plot. Geometric means of IC_{50} values from several muscle strips were obtained, with standard errors in logarithm

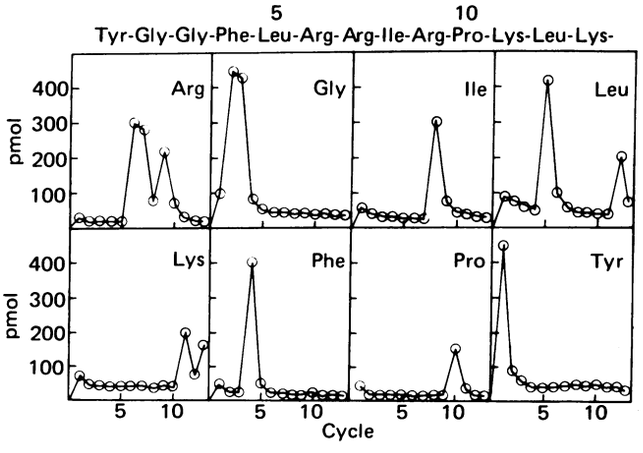

FIG. 1. Yields of phenylthiohydantoin amino acids from porcine dynorphin, run on NH_2-terminal sequenator. Each cycle was analyzed by high-performance liquid chromatography on a DuPont Zorbax CN column. Yields were calculated by comparison with a standard phenylthiohydantoin amino acid mixture. Analysis of the phenylthiohydantoin amino acids not shown here gave no cycles with levels significantly above background. The 14th and later cycles yielded too little material for unambiguous identification.

units. K_d values for naloxone as an antagonist were determined from the ratio of IC_{50} values in the presence and absence of a fixed naloxone concentration (9). The mouse vas deferens was used as described (10). IC_{50} and naloxone K_d values were determined as above. In both bioassays, volumes of methanol/HCl solvent up to 20 μl in the 5-ml tissue bath were without effect.

The radioreceptor binding assay used well-washed rat brain membranes that had been incubated for 30 min at 37°C with 100 mM NaCl to remove bound endogenous ligands (11, 12). Several radioligands (final concentration, 1 nM) and competing ligands were tested. Competing ligands were added to the membrane suspension in Tris pH 7.4 buffer at 23°C, and radioligand was added immediately thereafter. Assay volume was 500 μl. After incubation (1 hr, 23°C), the mixture was chilled, filtered, washed twice, and assayed for radioactivity. Displacement of radioligand by levallorphan (final concentration, 1 μM) was taken as measure of stereospecific saturable binding; this was usually about 70% of total binding. The (+) isomer, dextrallorphan, was without effect under these conditions. Methanol/HCl solvent, present in all incubation tubes in a volume of 5 μl, had no effect on control binding.

^{125}I-Labeled dynorphin-(1–13) was prepared by a modification of the method of Hunter and Greenwood (13) and separated from unreacted iodide on a Sephadex G-15 column with 0.1% bovine serum albumin in 0.25 M acetic acid. Peptide degradation *in vivo* and by rat brain membranes was estimated by a shift in the radioactivity peak on a Bio-Gel P-2 column (1.2 × 41 cm) with *n*-butanol/acetic acid/water, 2:1:4 (vol/vol).

For radioimmunoassay, two different antisera raised against [Leu]enkephalin in rabbits were used [these were gifts of Stanley Watson (University of Michigan) and Richard J. Miller (University of Chicago)]. They are the same antisera used in mapping [Leu]enkephalin immunoreactivity in the central nervous system (14, 15). Antisera were used at final dilutions required to bind about 30% of ^{125}I-labeled [Leu]enkephalin in the absence of a competing ligand. This radioligand was prepared by the chloramine-T method (13) and purified on DEAE-Sephadex. In each tube, 100 μl of peptide to be assayed was added to 100 μl of diluted antiserum, and the mixture was incubated for 48 hr at 4°C. Then, 100 μl of radioligand (≈5000 cpm) was added, and incubation was continued for another 24 hr. The incubation was terminated by adding 100 μl of normal rabbit plasma and 1.5 ml of 15.8% polyethylene glycol. After 10 min on ice, the mixture was centrifuged for 15 min at 4000 × *g*, the supernatant solution was aspirated, and the tube containing precipitate was placed in a gamma counter.

RESULTS

Typical opioid effects of dynorphin-(1-13) in the two bioassays are shown in Fig. 2. Table 1 summarizes data of several such experiments. In the guinea pig ileum preparation, the potency of the new peptide was, on the average, 730 times that of [Leu]enkephalin, 190 times that of normorphine, and 54 times that of β_c-endorphin. Its IC_{50} value in this tissue, 630 pM, makes it the most potent opioid peptide known; certain opiate alkaloid derivatives such as etorphine are more potent. The apparent dissociation constant (K_d) of naloxone as an antagonist was 13 times larger (i.e., affinity $^1/_{13}$th) with dynorphin-(1–13) as agonist than with either [Leu]enkephalin or normorphine. The magnitude of this difference in naloxone affinity is similar to that reported with ethylketocyclazocine (16). Standard doses of normorphine were tested on several preparations in the presence of a partially inhibiting concentration of dynorphin-(1–13), essentially as described by Kosterlitz and Watt (9). Simple additivity was always observed, indicating that the new peptide had no antagonist properties.

In the mouse vas, in which [Leu]enkephalin is outstandingly potent, dynorphin-(1–13) was about three times more potent. Again, as in the guinea pig ileum preparation, the naloxone K_d was much greater than with normorphine or [Leu]enkephalin as agonists.

The final column of Table 1 summarizes the relative potencies in the two bioassays. [Leu]Enkephalin was 20 times more potent in the vas, β_c-endorphin and normorphine were approximately equipotent in the two tissues, and dynorphin-(1–13) was 12 times more potent in the guinea pig ileum preparation.

The potencies of the two register-shift peptides in the guinea pig ileum preparation are summarized at bottom of Table 1. These peptides are identical to dynorphin-(1–13) except that in RS-1 a deletion of Arg^6 shifts the remaining COOH-terminal sequence to the left by about 0.5 nm and in RS-2 an insertion of Gly^6 shifts the remaining sequence to the right by the same amount. The loss of potency in both, compared with the parent peptide, makes it evident that the unique sequence embodied in dynorphin-(6–13), and the linear distance of this sequence from the [Leu]enkephalin sequence [dynorphin-(1–5)], are both critically important for maximal potency.

We studied the rate of reversal of twitch inhibition upon washing the guinea pig ileum preparation. First, a moderate degree of inhibition was established (range, 41–72%). Then, the bath fluid was replaced and twitch amplitude was measured again 3 min later. With this procedure, 21% and 22% recovery had been noted in two preparations of purified natural dynorphin. Mean (±SEM) recoveries for other agonists, with number of experiments in parentheses, were: synthetic dynorphin-(1–13), 13 ± 2% (7); β_c-endorphin, 45 ± 5% (4); normorphine, 78 ± 4% (7); [Leu]enkephalin, 101 ± 1% (4). Thus, the synthetic peptide was slow-reversing. Rate of reversal, which is influenced by the ligand–receptor dissociation rate constant, tends to be slower with more potent ligands.

Table 2 gives the results in the radioreceptor binding assay with washed rat brain membranes. Dynorphin-(1–13) was less potent than β_c-endorphin, approximately equipotent with normorphine, and much more potent than [Leu]enkephalin. With [^{3}H]naloxone as radioligand, the decreased affinity of dynorphin-(1–13) in the presence of 100 mM NaCl was typical of the "sodium shift" seen with agonists when an antagonist is radioligand. In some of the incubations, with the new peptide at approximately its IC_{50} concentration, ^{125}I-labeled dynorphin-(1–13) was added. At the end of the incubation, analysis of radioactivity on a molecular sieving gel column revealed that all of the original peptide had been degraded to material of lower apparent molecular weight. Thus, the observed potencies of the new peptide in this assay must be regarded as minima.

Radioimmunoassays were carried out with [Leu]enkephalin antisera no. 4 (Watson) and no. 198 (Miller), both of which had been used for immunohistochemical localization of [Leu]enkaphalin (14, 15). Complete concentration–inhibition curves were run, with ^{125}I-labeled [Leu]enkephalin as radioligand and [Leu]enkephalin and dynorphin-(1–13) as competing ligands. Parallel lines were obtained, with a slope in the midrange consistent with a simple adsorption isotherm. Crossreactivity of dynorphin-(1–13), computed from the ratio of IC_{50} values (means of two experiments), was 12% with antiserum no. 4 and 8% with antiserum no. 198.

In preliminary experiments (unpublished observations) we observed catalepsy and analgesia in some rats a few minutes after injection of 50 nmol (80 μg) of dynorphin-(1–13) into the lateral ventricle. Investigations on the central effects of the peptide will be complicated by its rapid degradation *in vivo*.

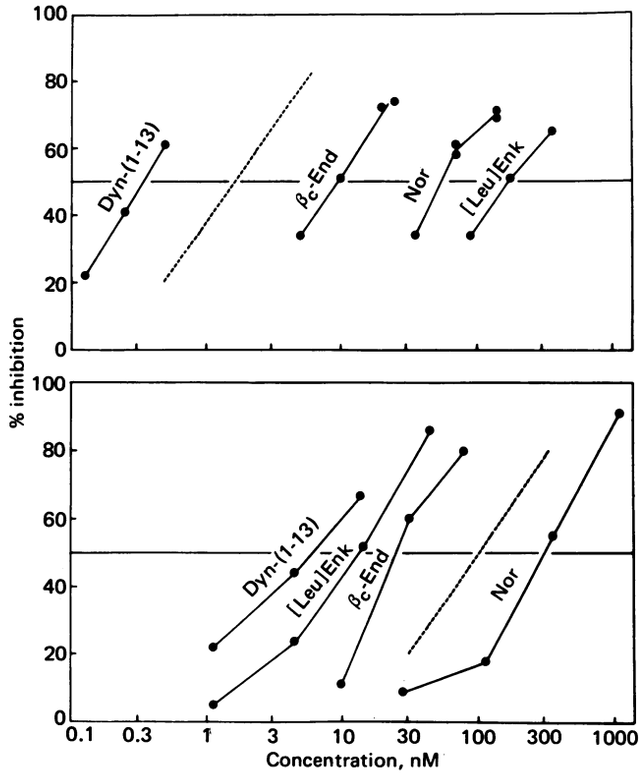

FIG. 2. Typical dose–response relationships for dynorphin-(1–13) and other agonists in a single preparation each of guinea pig ileum longitudinal muscle strip (*Upper*) and mouse vas deferens (*Lower*). Broken line represents theoretical slope at midpoint of the fractional occupancy curve based on the mass law equation $[(R)(X)/(RX) = K]$ and the Michaelis–Menten assumption that a negligible fraction of total ligand is bound to receptor sites. Dyn-(1-13), dynorphin-(1–13); β_c-End, camel β-endorphin; Nor, normorphine; [Leu]Enk, [Leu]-enkephalin.

Ten minutes after injection of ^{125}I-labeled peptide, extraction of brain [methanol/1 M HCl, 1:1 (vol/vol); 100°C; analysis on Bio-Gel P-2], revealed that most of the peptide had already been degraded.

DISCUSSION

Dynorphin-(1–13) has been shown here to have typical opioid activity in several different assay systems. It inhibits the electrically stimulated twitch of the guinea pig ileum longitudinal muscle, and this effect is blocked and reversed by the specific opiate antagonist naloxone. The peptide is extraordinarily potent—about 700 times more potent than [Leu]enkephalin, 200 times more potent than normorphine, and 50 times more potent than β_c-endorphin. In this tissue preparation, determination of potency is not complicated by degradation because any loss of twitch inhibition can be observed directly. Even with [Leu]enkephalin, this rarely occurred. Naloxone is $^1/_{13}$th as potent in blocking this peptide as it is in blocking normorphine or [Leu]enkephalin, but complete blockade is nevertheless obtained at high enough concentrations. In additivity experiments, the peptide had no antagonist properties in this tissue.

In the mouse vas deferens, in which degradation is also not seen, the peptide is about 3 times more potent than [Leu]enkephalin. A major difference between the opiate receptor(s) in guinea pig ileum and in mouse vas deferens is the differential sensitivity of the latter to [Leu]enkephalin. Thus, dynorphin-(1–13) presents an interesting counterpart to [Leu]enkephalin

Table 1. Effects of dynorphin-(1–13) in two bioassays

	Guinea pig ileum myenteric plexus longitudinal muscle preparation			Mouse vas deferens			
	IC_{50}, nM	SEM, log units	Naloxone $K_d \pm$ SEM, nM	IC_{50}, nM	SEM, log units	Naloxone $K_d \pm$ SEM, nM	Potency ratio, mouse vas/guinea pig ileum
Dynorphin-(1–13)	0.63	0.07 (18)	33 ± 4	7.5	0.06 (8)	120 ± 49	0.08
[Leu]Enkephalin	460	0.09 (18)	2.5 ± 0.1	23	0.10 (8)	40 ± 10	20
β_c-Endorphin	34	0.11 (4)	—	24;21	— (2)	—	1.5
Normorphine	120	0.06 (18)	2.1 ± 0.2	230	0.11 (8)	12 ± 6	0.52
RS-1	7.5	0.07 (7)	20 ± 4	—	—	—	—
RS-2	4.8	0.08 (8)	15 ± 1	—	—	—	—

IC_{50} values were obtained from assays spanning the 50% inhibition response, plotted as in Fig. 1. Naloxone K_d is the apparent dissociation constant of the antagonist, computed from the equation $K_d = C/(DR - 1)$, derived from the mass law for competitive antagonism, in which C is concentration of naloxone (here, 100 nM), and DR is dose ratio of agonist [the ratio of IC_{50} doses in the presence and absence of the antagonist (9)]. The same procedures were followed with the mouse vas deferens. IC_{50} values are geometric means; SEM are in log units; number of tissue preparations is shown in parentheses. K_d determinations were based on four tissue preparations. RS-1 is des-Arg^6-dynorphin-(1–13); RS-2 has Gly^6 inserted in dynorphin-(1–13).

in that its potency is relatively much greater in the guinea pig ileum than in the mouse vas.

Dynorphin-(1–13) competes with opiate alkaloid and opioid peptide radioligands for stereospecific binding to rat brain membranes. It is not exceptionally potent in this assay, but that may be due to its demonstrated rapid degradation by a membrane-bound enzyme. Its sodium shift is typical of that seen with agonists such as normorphine and β_c-endorphin.

Artificial enhancement of the potency of enkephalins has been achieved primarily through synthesis of analogues (such as the D-Ala^2 derivatives) less subject to attack by degradative enzymes (17). The greater potency of β-endorphin than of [Met]enkephalin [β-endorphin-(1–5)] is also associated with greater metabolic stability (18). A different basis for high potency is evident in the structure of dynorphin-(1–13). The sequence -Arg-Arg-Ile-Arg-Pro-Lys-Leu-Lys-OH, as a COOH-terminal extension of [Leu]enkephalin, enhances the potency of that pentapeptide by nearly 3 orders of magnitude in the guinea pig ileum preparation and also results in a greatly decreased apparent affinity of naloxone as an antagonist. If this tissue contains more than one type of opiate receptor (16, 19, 20), it follows that the [Leu]enkephalin receptor is different from the dynorphin receptor with respect to affinity both for these peptide ligands and for naloxone.

The dynorphin receptor evidently has an extensive binding site, of which only one part interacts with enkephalin pentapeptides or the structurally similar opiate alkaloids. Thus, investigations using compact opiate alkaloids or small opioid peptides as ligands have presumably probed only a small part of this receptor—what might be called the *enkephalin pocket*. Binding studies with radiolabeled dynorphin should be of interest in characterizing the entire binding site on the receptor.

Table 2. Effects of dynorphin-(1–13) in the radioreceptor binding assay

	IC_{50} values with different radioligands, nM			
	[^{3}H]Naloxone			
Competing ligand	−Na	+Na	[^{3}H]DADLE	[^{3}H]DHM
Dynorphin-(1–13)	14;22	145;165	43*	16
[Leu]Enkephalin	6000*	—	400*	2100*
β_c-Endorphin	1.4;1.9	34;40	2.1	1.8
Normorphine	21	260	32*	5.0

The assay used washed membranes from rat brain homogenate and 1-hr incubation at 23°C. Data are IC_{50} values from complete log dose-inhibition curves for inhibition of stereospecific binding of the various radioligands. Total stereospecific binding was determined with levallorphan (1 μM) as competing ligand; dextrallorphan (1 μM) was without effect. DADLE, [D-Ala^2,D-Leu^5]enkephalin; DHM, dihydromorphine.

* These slopes were unusually shallow; all other slopes were compatible with the theoretical slope expected from the mass law equation.

Recently, a partial sequence was reported for α-neo-endorphin, an opioid peptide from the porcine hypothalamus (21). Its sequence beyond residue 6 differs from that of dynorphin-(1–13), its composition is incompatible with that of dynorphin-(1–13), and it is much less potent. The functional relationship of these two [Leu]enkephalin-containing peptides remains to be elucidated.

The following evidence suggests that the synthetic tridecapeptide studied here is identical to the NH_2-terminal portion of the natural peptide from porcine pituitary that we purified.

(*i*) The sequence of the 13 residues was determined by a microsequencing technique, despite the presence of a NH_2-blocked contaminant and the lack of sufficient material for classical proofs of identity. The finding of [Leu]enkephalin at the NH_2 terminus, followed by Arg-Arg, lends plausibility to the result.

(*ii*) The previously reported resistance of the biologic activity of the natural peptide to cyanogen bromide is explained by the absence of methionine from the NH_2-terminal sequence.

(*iii*) The previously reported extreme basicity is explained by the presence of arginine residues.

(*iv*) The previously reported virtually complete destruction of biologic activity by trypsin is explained by the 2000-fold greater potency of the tridecapeptide than of its biologically active tryptic fragment, [Arg^6-Leu]enkephalin (21).

(*v*) The previously reported slow reversal of inhibition on washing of the guinea pig ileum preparation is seen also with the synthetic tridecapeptide.

(*vi*) The synthetic tridecapeptide has approximately the same molar potency as the natural peptide in the guinea pig ileum preparation.

(*vii*) The remarkably high potency depends upon an exact linear distance of the COOH-terminal extension from the NH_2-terminal [Leu]enkephalin; thus, the sequence of the tridecapeptide appears to be uniquely important for potency.

The physiologic role of the enkephalin pentapeptides needs to be reexamined. It is unlikely that dynorphin is a mere precursor of [Leu]enkephalin because it is already so much more

potent than the pentapeptide. It is attractive to suppose that, at some sites, dynorphin is a physiologically active hormone or neurotransmitter. At such sites, the physiologic action might be terminated by cleavage to smaller fragments such as [Leu]-enkephalin or [Arg^6-Leu]enkephalin. Inasmuch as we have found significant immunoreactivity of dynorphin-(1–13) with specific [Leu]enkephalin antibodies, it is possible that some of the "[Leu]enkephalin" mapped by immunohistofluorescence in the nervous system could actually be dynorphin.

Note Added in Proof. The sequence reported here for dynorphin-(1-13) has been confirmed in an independent isolation from another batch of melanotropin concentrate.

None of this work could have been done without the cooperation of Dr. J. D. Fisher (Armour Pharmaceuticals, Kankakee, IL) whose foresight was responsible for stockpiling byproducts of commercial pituitary hormone production and who furnished the melanotropin concentrate that was our starting material. Drs. Stanley Watson and Richard J. Miller kindly supplied samples of their [Leu]enkephalin antisera. Asha Naidu provided technical assistance of the highest quality throughout this project. We thank Dr. Maureen Ross and Fanny Liu for the radioimmunoassays, Dr. Barbara Herman and Clarence Omoto for their participation in the preliminary behavioral experiments, Dr. Vartan Ghazarossian and Keiko Otsu for the degradation studies, Patricia Lowery for some of the assays, and Dr. Brian M. Cox for helpful comment and criticism. The work was supported by Grant DA-1199 from the National Institute on Drug Abuse and Grant GM-06965 from the National Institutes of Health.

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